

REMARKS

A substitute sequence listing and substitute Figures are submitted herewith to correct the nucleotide and amino acid sequences originally presented in the ICE-LAP 3 sequences (shown in the sequence listing as SEQ ID NOs:1 and 2 and in Figures 1 and 3). These changes do not introduce new matter because the correct sequence was inherent to the originally sequenced plasmid clone in actual possession of the Applicants before the time the captioned application was filed. The analysis needed to determine the complete and correct sequences were well within the skill of the ordinary artisan, and such analysis would not have required undue experimentation.

These same corrections to the Figures were submitted and entered in related Application No: 08/462,969 (now U.S. Patent No. 6,087,150). Attached hereto as Exhibit A is a copy of the Amendment and accompanying Declaration of Craig Rosen under 37 C.F.R. § 1.132 with Exhibits A-E thereto requesting correction of the sequence listing and figures for the aforementioned application. In this amendment, Applicants demonstrate that the corrected sequences are inherently present in the deposited material and, because Applicants have demonstrated "chain of custody" for the material originally sequenced and the resequenced material, correction of the originally presented sequence herein is not new matter. Furthermore, support for the correction of such sequencing errors can be found in the specification at page 4, lines 20 – 24 which state:

"Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figures 1 and 2 are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%."

The corrections to SEQ ID NO:1 submitted herein do not constitute a change of greater than 3%, hence the corrected sequence is fully supported by the specification and is not new matter.

Further, the Sequence Listing filed herewith includes sequences (SEQ ID NOs:5-12) corresponding to the primer sequences set forth in the examples of the instant application. The specification is amended as set forth above to insert the appropriate SEQ ID NOs at the appropriate locations for such primer sequences.

The specification has been amended to add the cross reference to the related application.

The specification was amended to reflect the drawing designations provided for in the formal drawings.

The specification was further amended on page 5 to correct the location of the pentapeptide QACRG in SEQ ID NO:2.

The specification was further amended on page 5 to correct the spelling of "homolog".

Accordingly, no new matter has been added herein.

Statements Under 37 C.F.R. § 1.825(a) and (b)

In accordance with 37 C.F.R. § 1.825(a), the undersigned attorney for Applicants hereby states that sequence information contained in the Substitute Sequence Listing submitted herewith is completely supported by the specification as originally filed, as detailed above, and no new matter has been introduced.

In accordance with 37 C.F.R. § 1.825(b), the undersigned attorney for Applicants hereby states that the information in the paper copy of the Substitute Sequence Listing submitted herewith is identical to the information contained in the computer readable form of the Substitute Sequence Listing submitted herewith.

SUMMARY

Applicants respectfully request entry of the amendments and remarks contained in this paper. If the Examiner believes that a telephone conference would help facilitate entry of the herein amendments, she is encouraged to call Applicants' representative at the telephone number listed below.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: He et al.

Attorney Docket No.: PF140C2

Application Serial No.: Unassigned

Art Unit: Unassigned

Filed: Herewith

Examiner: Unassigned

Title: Interleukin-1 Beta Converting Enzyme
Like Apoptosis Protease-3 and 4

VERSION OF AMENDMENTS WITH MARKINGS
TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at page 4, line 7, has been amended as follows:

Figures 1A-B shows the cDNA and corresponding deduced amino acid sequence of ICE-LAP-3. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue), and the standard one-letter abbreviation for amino acids is used.

Paragraph beginning at page 4, line 12, has been amended as follows:

Figures 2A-B shows the cDNA and corresponding deduced amino acid sequence of ICE-LAP-4. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue).

Paragraph beginning at page 4, line 16, has been amended as follows:

Figures 3A-C shows an amino acid sequence comparison between ICE-LAP-3, ICE-LAP-4, human ICE and the C. elegans cell death gene ced-3. Shaded areas represent amino acid matches between the different sequences.

Paragraph beginning at page 4, line 20, has been amended as follows:

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Sequencing inaccuracies are a common problem when attempting to determine polynucleotide sequences. Accordingly, the sequences of Figures 1A-B and 2A-B are based on several sequencing runs and the sequencing accuracy is considered to be at least 97%.

Paragraph beginning at page 4, line 25, has been amended as follows:

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode the mature polypeptides having the deduced amino acid sequence of Figures 1A-B and 2A-B or for the mature polypeptide encoded by the cDNA of the clones deposited as ATCC Deposit No. 75875 encoding ICE-LAP-3, and ATCC Deposit No. 75873 encoding ICE-LAP-4, which were deposited August 25, 1994.

Paragraph beginning at page 4, line 33, has been amended as follows:

The polynucleotide encoding ICE-LAP-3 can be detected from human prostate, human endometrial tumor, human pancreatic tumor, human adrenal gland tumor and human tonsil. The full-length encoding ICE-LAP-3 was discovered in a cDNA library derived from human endometrial tumor. It is structurally related to the Interleukin-1 converting enzyme family. It contains an open reading frame encoding a protein of approximately 341 amino acid residues. The protein exhibits the highest degree of homology to *C. elegans* cell death gene *ced-3* which is a ~~homolog~~ homolog of human interleukin-1 converting enzyme, with 68 % similarity and 43 % identity over the entire amino acid sequence. It should be pointed out that the pentapeptide QACRG is conserved and is located at amino acid position ~~259-263~~ 184-188.

Paragraph beginning at page 5, line 21, has been amended as follows:

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encode the mature polypeptides may be identical to the coding sequence shown in Figures 1A-B and 2A-B or that of the deposited clones or may be a different coding sequence which coding sequence, as a

result of the redundancy or degeneracy of the genetic code, encode the same mature polypeptides, and derivatives thereof, as the DNA of Figures 1 A-B and 2 A-B or the deposited cDNA.

Paragraph beginning at page 5, line 34, has been amended as follows:

The polynucleotides which encode for the mature polypeptides of Figures 1 A-B and 2 A-B or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Paragraph beginning at page 6, line 10, has been amended as follows:

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figures 1 A-B and 2 A-B or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be naturally occurring allelic variants of the polynucleotides or non-naturally occurring variants of the polynucleotides.

Paragraph beginning at page 6, line 18, has been amended as follows:

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1 A-B and 2 A-B or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1 A-B and 2 A-B or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

Paragraph beginning at page 6, line 27, has been amended as follows:

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in

Figures 1A-B and 2A-B or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of nucleotides, which does not substantially alter the function of the encoded polypeptides. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues.

Paragraph beginning at page 7, line 11, has been amended as follows:

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNAs of Figures 1A-B and 2A-B or the deposited cDNA(s).

Paragraph beginning at page 8, line 4, has been amended as follows:

The present invention further relates to ICE-LAP-3 and 4 polypeptides which have the deduced amino acid sequence of Figures 1A-B and 2A-B or which has the amino acid sequence encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptides.

Paragraph beginning at page 8, line 9, has been amended as follows:

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1A-B and 2A-B or that encoded by the deposited cDNA, means polypeptides which retain essentially the same biological function or activity as such polypeptides, and wherein derivatives include

polypeptides with enhanced or reduced biological function. An analog includes a proprotein portion to produce active mature polypeptides.

Paragraph beginning at page 8, line 20, has been amended as follows:

The fragment, derivative or analog of the polypeptides of Figures 1A-B and 2A-C or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the mature polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Paragraph beginning at page 26, line 11, has been amended as follows:

--The DNA sequence encoding ICE-LAP-3, ATCC # 75875, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-3 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-3 gene. Additional nucleotides corresponding to ICE-LAP-3 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGCGTGCGGGGACACGGGTC 3' (SEQ ID NO:5) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATCATTACCCCTGGTGGAGGAT 3' (SEQ ID NO:6) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-3. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an ITPG-regulatable

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promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. PQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform *E. coli* available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalactopyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-3 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-3 (95% pure) is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.—

Paragraph beginning at page 28, line 1, has been amended as follows:

-- The DNA sequence encoding ICE-LAP-4, ATCC # 75873, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-4 protein (minus the signal peptide

sequence) and the vector sequences 3' to the ICE-LAP-4 gene. Additional nucleotides corresponding to ICE-LAP-4 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGGAGAACTGAAACTCA 3' (SEQ ID NO:7) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATTAGTGATAAAAAATAGAGTTC 3' (SEQ ID NO:8) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-4. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform *E. coli* available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalactopyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-4 is purified from

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this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-4 (95% pure is eluted from the column in 6 molar guanidine HCL pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.--

Paragraph beginning at page 30, line 7, has been amended as follows:

-- The DNA sequence encoding for ICE-LAP-3, ATCC # 75875, was constructed by PCR on the full-length ICE-LAP-3 using two primers: the 5' primer 5' GACTATGCGTGCGGGGACACGG 3' (SEQ ID NO:9) contains the ICE-LAP-3 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-3 coding sequence starting from the initiation codon; the 3' sequence 5'

AATCAAGCGTAGTCTGGGACGTCGTATGGGTATTACCCCTGGTGGA GGATTTG 3' (SEQ ID NO:10) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-3 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-3 coding sequence followed by HA tag fused in frame, and a translation termination stop codon next to the HA tag. The PCR amplified DNA fragment was ligated with pcDNA1/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-3, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-3 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory

Press, (1988)). Cells were labelled for 8 hours with ³⁵S-cysteine two days post tranfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

Paragraph beginning at page 32, line 20, has been amended as follows:

-- The DNA sequence encoding for ICE-LAP-4, ATCC # 75873, was constructed by PCR on the full-length ICE-LAP-4 using two primers: the 5' primer 5' ACCATGGAGAACACTGAAAAC 3' (SEQ ID NO:11) contains the ICE-LAP-4 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-4 coding sequence starting from the initiation codon; the 3' sequence 5'

AATCAAGCGTAGTCTGGGACGTCGGTATGGGTAGTGATAAAAATA GAGTTCTTT 3' (SEQ ID NO:12) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-4 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-4 coding sequence followed by HA tag fused in frame, and a translation termination stop codon next to the HA tag. The PCR amplified DNA fragment was ligated with pcDNA1/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-4, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-4 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ³⁵S-cysteine two days post tranfection. Culture media were then collected and cells were lysed with

detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. Et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.--

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